REMARKS

Claims 3-5 and 19-21 remain in the present application. No amendments have been made to claims 3-5. The Office Action of September 13, 2001, has been carefully studied. It is believed that all of the claims are patentable.

Specification

The specification has been corrected to correct the typographical error in spelling "animal" and to give the full name of the compounds or substances which are the subject of the abbreviations on pages 7 and 8.

Rejections under 35 U.S.C. 112

Claims 3 and 5 are rejected under 35 U.S.C. 112, first paragraph, the Examiner alleging that the specification does not reasonably provide enablement for fungal species other than *Stachybotrys chartarum*.

This rejection is respectfully traversed. As disclosed in the specification of the present application, paragraph 007, many fungi do not produce normal antibodies to the body or the mycelium of the organism in humans. Therefore, it is not possible to identify exposure to these fungi by ordinary assay means. Although hemolysins have been obtained and purified from many bacterial strains (*cf.* citations on page 3 of the

application), there have previously been no reports of **isolating active** hemolysins from fungi.

The present inventor has now discovered that a number of fungal strains produce fungal hemolysins, and that these hemolysins can be isolated by using the culture supernatant or filtrate rather than an homogenate. This is important because the fungal hemolysins are glycoslyated, secretory proteins, and the active form of this protein is in the culture filtrate/supernatant. These active fungal hemolysins can be used to determine if an organism has been exposed to the fungus which produces the particular hemolysin.

Once it had been discovered that it was possible to isolate active forms of hemolysins from fungi, one skilled in the art could easily extract hemolysins from fungi other than *Stachybotrys chartarum* and produce antibodies thereto without undue experimentation. Of course, only those fungi that do produce hemolysins as a secretory protein are relevant. In fact, as can be seen from the attached Vesper et al. preprint, even some strains of *Stachybotrys* do not produce stachylysin under these conditions, which may make the threat of these non-producing strains to humans and animals fundamentally different.

Once it has been determined that a fungus produces hemolysin, and it is known to obtain the active form of the hemolysin from the culture supernatant or filtrate, the remainder of the purification is merely a conventional purification process used for protein purification, such as in "Guide to Protein Purification" in *Methods in Enzymology*, vol. 182. Any protein biochemist, *i.e.*, one skilled in this particular art, could effect the purification one that person knew the step of how to produce the relevant hemolysin. "In fact, the attached preprint even provides improvements in the purification step that make the purification even easier. These changes are merely variations of well known procedures, again known to those skilled in this art.

The claims of the present application are directed to determining if a mammal has been exposed to a fungus which produces hemolysin. Before the present inventor's discovery that some fungi produce fungal hemolysins, and that the active form of these fungal hemolysins could be isolated, there was no way to identify if a mammal had been exposed to a hemolysin-producing fungus. With the knowledge that some fungi produce hemolysin which can be isolated, one skilled in the art, with the information provide in the present specification, can, without undue experimentation,

isolate the fungal hemolysin, produce antibodies thereto, and use these antibodies to determine the presence in a sample of antigens to the particular fungal hemolysin.

The method of the present invention depends upon analysis of an active form of fungal hemolysin, a glycosylated, secretory protein. The active form of the protein is in the culture filtrate/supernatant, as described in the attached Vesper et al. preprint. The hemolysin protein extracted from the fungal cells, which forms the basis of the presently claimed assays, is not the active form of the protein, because glycosylated proteins are processed during secretion and change their physical structure, chemical composition, immunological characteristics, and, most importantly, their biological activity.

While the examples are based on extracting hemolysin from *Stachybotrys chartarum,* the present invention relates to purifying fungal hemolysin proteins so that these hemolysin proteins can be used to produce antibodies to demonstrate exposure to fungi which produce hemolysin. The present application teaches one skilled in the art how to obtain the active form of the hemolysin from any fungus that produces fungal hemolysin.

It should be appreciated that this process is

relevant only to those fungi that do produce hemolysins as a secretory protein. In fact, it is now known that even some strains of *Stachybotrys* do not produce stachylysin under some conditions, which may make the threat of these non-producing strains to humans and animals fundamentally different.

Once it had been discovered that some fungi produce hemolysin proteins, and that these fungal hemolysins could be obtained in the active form, one could then use conventional purification steps for obtaining antibodies to the hemolysins. What is important to the present invention, then, is the discovery that an active form of the hemolysin can be obtained. Once the active form of the fungal hemolysin is obtained, *i.e.*, from the filtrate/culture supernatant, purifying the hemolysin proteins and obtaining antibodies thereto is within the capabilities of one skilled in this art, which would not require undue experimentation to perfect for other fungi. Any protein biochemist could conduct the purification one he or she knows how to produce the relevant active form of the hemolysin, and then produce antibodies thereto.

Art Rejections

Claims 3-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Ishida et al. The Examiner

alleges that Ishida et al. teach a method for enzyme immunoassay of *Aspergillus* hemolysin by cheimluminescence reaction of luminol-peroxidase. This paper is said to indicate that *Aspergillus* hemolysin can be accurately determined within the range of 10-100 pg by this method.

This rejection is respectfully traversed. Ishida et al. merely disclose that Asp-hemolysin can be determined. However, what is significant about the present invention is that the present inventor has discovered that a number of fungi produce hemolysins and that these hemolysins can be isolated in the active form. Because of this discovery, it is now possible to isolate these fungal hemolysins and use them to produce antibodies which can be used to determine if a mammal has been exposed to a hemolysin-producing fungus. It should be noted that the present application discloses isolation of fungal hemolysin beginning at paragraph 0025.

Submitted herewith is a copy of Yokota et al., *Microbiol. Immunol.* **21(1)**: 11-22, 1977, in which the authors describe obtaining a hemolytic toxin from *Asp. fumigatus*. In this method, the crude toxin is obtained from culture filtrates or mycelia of *Asp.* funigatus, and it was noted that the two hemolysins obtained were the same. On page 20, Yokota et al. note that the hemolysin contains 0.8% sugar as glucose, so that the hemolysin is

considered to be a sugar containing protein. It is assumed that this is the hemolysin that Ishida et al. used. There is absolutely nothing in Ishida et al. that teaches or even suggests that the Asp-hemolysin is obtained in the active form solely from the filtrate/supernatant. It is this active form of the hemolysin that is used in the present invention to determine if a mammal has been exposed to a hemolysin-producing fungus. While Ishida et al. disclose that Asp-hemolysin can be determined by enzyme immunoassay, there is no teaching that there is a difference between the active form of the hemolysin and the inactive form. As noted above, the fungal hemolysins are glycosylated, secretory proteins and the active form of the present in is round only in the culture filtrate/supernatant. The hemolysin protein extracted from the fungal cells is not the active form because the glycolsyated proteins are processed during secretion and change their physical structure, chemical composition, immunological characteristics, and, most importantly, their biological activity.

The present invention is directed to a test using only the active or relevant form of the protein. Thus, if an immunological procedure is used to generate antibodies, the antibodies are generated only to the

active form the protein, which is the form which is relevant for health and diagnostic application. If the whole homogenate of the fungal cells is used, and not solely the filtrate/supernatant of the culture, for antibody production, this could result in antibody reagent production which is irrelevant to the active form of the hemolysin, and thus may not be diagnostically useful.

There is no recognition in Ishida et al. that the Asp-hemolysin was the active form. If the Asp-hemolysin is not the active form, it is useless for diagnostic purposes.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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IN THE SPECIFICATION

Page 7, please amend paragraph [0028] as follows:

[0028] The hemolysin protein as obtained above was conjugated to KLH **(key hole limpet hemocyanin)** as a carrier, using glutaraldehyde chemistry, linking amines of the protein and the carrier. This preparation was injected into animals as an immunogen. However, fungal hemolysins proteins need not be conjugated for use in a vaccine.

Page 8, please amend paragraph [0031] as follows:

[0031] An ELISA assay was conducted by coating peptide onto microliter wells and reacting the peptide with dilutions of antibody or anti-hemolysin/ALP **(alkaline phosphate)**, then with Gxrabbit IgG (h&l) **(goat anti-rabbit Ig, both heavy and light chains)**/HRP **(horseradish peroxidase)** (not for ALP conjugate) followed by TMB **(tetramethyl benzidine)**/H_2O_2 or BCIP **(4-bromo, 4-chloro, 3-indol phosphate)** substrate. The following table indicates the dilutions of the antibody solutions that produced an OD of 1.0 in this assay:

Specificity Antibody	Antibody Prep	Antisera	Dilution A. **(affinity)** P. **(purified)** Antibody
Hemolysin	7980/2	1/41,000	1/27,160

Page 8, please amend paragraph [0032] as follows:

[0032] These antibodies to fungal hemolysin can be used in a conventional immunoassay such as an ELISA to determine ~~of~~ **if** one has been exposed to strains of fungi which produce hemolysin. The hemolysin protein itself can be used to determine if one has produced antibodies in response to exposure to the fungus.

Page 12, please amend paragraph [0044] as follows:

[0044] The purified polypeptide fragments thus obtained can be tested to determine their immunogenicity and specificity. Briefly, various concentrations of a putative immunogenically specific fragment are prepared and administered to an ~~anima~~ **animal**, and the immunological response (*e.g.*, the production of antibodies or cell mediated immunity) of an animal to each concentration is determined. The amounts of antigen administered depend on the subject, the condition of the subject, the size of the subject, etc. Thereafter, an animal so inoculated with the antigen can be exposure to

the fungus to test the potential vaccine effect of the specific immunogenic fragment. The specificity of a putative immunogenic fragment can be ascertained by testing sera, other fluids, or lymphocytes from the inoculated animal for cross reactivity with other closely related fungi.